HLA-A, B, C, DRB1, DQB1 matched siblings (29), matched unrelated donors (22), or unrelated donors mismatched for one HLA antigen (6), homozygous mismatch (1), one HLA allele (2), or two HLA alleles (1). Fludarabine 40 mg/m^2 was given intravenously daily for four days, with each infusion followed immediately by intravenous busulfan. The dose of busulfan for days 1 and 2 was 130 mg/m^2. Pharmacokinetic analysis was performed after the first infusion of busulfan; in 59 pts, the goal was to adjust busulfan doses for days 3 and 4 to achieve an average targeted Css level of 800-1000 ng/ml. Levels were drawn incorrectly in 4 of these pts and doses were not changed. Thirty-five (59%) pts had their doses adjusted, increased in 27 and decreased in 8, while 20 pts had Css within the desired range without adjustment. Patients received tacrolimus and standard doses of methotrexate for GVHD prophylaxis, with the exception of five patients. Engraftment occurred in 58 (95%) pts. Thirty (64%) of 47 pts followed for at least 100 days experienced acute GVHD requiring treatment. Six pts have died of transplant-related complications and 7 pts have failed to achieve remission or have relapsed. Median follow-up is 174 days (range 26-448 days). The 100-day K-M estimate of survival for the whole cohort is 92%, and event-free survival 88%. The 100-day mortality in this study compares well with the 100-day mortality reported to the IBMTR for patients with AML, ALL, MDS, and CML transplanted from either HLA-matched siblings or unrelated donors. These preliminary results indicate that tBuFlu is a promising myeloablative regimen that can be utilized in older patients with low early treatment-related mortality.

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LACK OF IMMUNE BARRIER TO ALLOGENEIC HEMATOPOIETIC STEM CELL ENGRAFTMENT IN T, B, AND NK CELL DEFICIENT (RAG2ΓC$^{-/-}$B6) MICE

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The way that allogeneic hematopoietic stem cells (HSC) resist engraftment is not completely understood. Natural killer cells (NK) and lymphocytes are thought to mediate the allograft barrier in mice that are mismatched at the major histocompatibility complex (MHC). The clearing of niche space is also thought to be required for donor cell engraftment. Here we attempt to dissect the relative contribution of these host elements to hematopoietic resistance using genetically defective Rag2$^{-/-}$ (*H2^b*) mice lacking T and B cells, or Rag2γc$^{-/-}$ (*H2^b*) mice lacking in T, B, and NK cells as recipients. We have previously shown that HSCs encounter greater resistance to engraftment when compared to unfractionated bone marrow (BM), and the resistance can be quantitated by titrating numbers of HSCs needed to rescue lethally irradiated recipients. Rescue of syngeneic or CD45 congenic recipients requires only 200 HSCs, whereas higher HSC doses are required as the genetic disparity increases. In this study, radioresistant MHC-mismatched AKR/J (*H2^k*) HSCs were transplanted into lethally irradiated (950 cGy) B6.WT (*H2^b*). All B6.WT mice died of hematopoietic failure despite attempted rescue with 1000 AKR/J HSC. No significant improvement in engraftment was observed in Rag2$^{-/-}$ mice when compared to B6.WT mice. However, an impressive difference was noted in the Rag2γc$^{-/-}$ mice, in which the immune barrier completely disappeared. A dose of 300 HSC rescued all irradiated Rag2γc$^{-/-}$ mice and even 200 AKR HSC, an amount equivalent to a congenic dose rescued 100% of recipients. We then sought to determine if engraftment could be achieved using non-myeloablative conditioning, or no radiation at all. Rag2γc$^{-/-}$ recipients of 6000 AKR/J HSCs treated with 500 cGy-300 cGy resulted in 100% donor engraftment. Additionally, unconditioned Rag2γc$^{-/-}$ also engrafted since 10-20% of donor AKR/J granulocytes were detected. In contrast, unconditioned Rag2$^{-/-}$ mice showed no evidence of donor cell engraftment. We also studied the trafficking of allogeneic FVB (H-2^q) HSC in irradiated versus unirradiated Rag2γc$^{-/-}$ (H-2^d) recipients by in vivo bioluminescence imaging. HSC were observed to enter the marrow space of irradiated mice within minutes following infusion, whereas unirradiated mice demonstrated no luciferase signal until day +5 post-infusion. We conclude that Rag2γc$^{-/-}$ mice have a profound reduction in the immune barrier to allogeneic HSC engraftment and, that in irradiated mice, HSC rapidly enter the marrow.

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POST-TRANSPLANTATION CYCLOPHOSPHAMIDE (Cy) AS A SINGLE AGENT FOR GVHD PROPHYLAXIS AFTER HLA MATCHED RELATED AND UNRELATED BONE MARROW TRANSPLANTATION

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In animal models of BMT, a properly timed high dose of Cy post-BMT selectively eliminates host-versus-graft and graft-versus-host reactive T cells, thereby preventing graft rejection and reducing GVHD. We hypothesized that high dose posttransplant Cy (50 mg/kg IV) administered on days +3 and +4 after BuCy conditioning may be effective in preventing GVHD and can limit, or entirely eliminate the need for, standard postgrafting immunosuppression. This should lessen immunosuppression and allow early institution of additional posttransplant immunotherapy such as DLI. 28 patients with advanced hematologic malignancies were conditioned with busulfan (PO or IV) on days −7 to 3 and Cy on days −2 and −1, transplanted with non–T cell–depleted marrow, and treated with Cy on days +3 and +4 as only postgrafting immunosuppression. 15 patients (median age 41 years) were allografted with bone marrow from HLA-identical siblings. Time to neutrophil (>500/μl) and platelet (>20000/μl, untransfused) engraftment was 22 and 31 days, respectively. One patient experienced secondary graft failure and was successfully rescued. Acute GVHD occurred in 7/15 patients at a median of 43 days after transplantation (range 20-68 days) and was exclusively grades I (2 patients) and II (5 patients). All 7 patients with GVHD responded completely to standard therapy (steroids only or steroids + FK-506) and all of them were successfully rapidly weaned from all immunosuppressive agents. With a median follow-up of 290 days (range 50-380), 10/15 patients are alive (all 5 patients died of relapsed disease) of which 7 are in remission. 13 patients (median age 41 years) received bone marrow from HLA-matched unrelated donors. Primary graft failure occurred in 2 recipients of unrelated marrow, and was fatal in one. One patient died from VOD. Time to neutrophil and platelet engraftment was 25 and 71 days, respectively. Of the 11 patients that engrafted, 1 developed grade I, 4 developed grade II and 1 developed grade III acute GVHD. All of them rapidly responded to standard therapy. From an overall survival perspective, 10/13 patients are alive of which 6 are in remission, with a median follow-up of 290 days (range 75-430). This preliminary analysis suggests that high dose post-transplantation Cy is effective as a single agent in the prophylaxis of severe GVHD after myeloablative conditioning and HLA-matched related BMT and should be studied in patients with standard risk hematologic malignancies.

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OUTCOME OF ALTERNATIVE DONOR TRANSPLANTATION FOR SEVERE APLASTIC ANEMIA CAN BE COMPARABLE TO OUTCOME WITH MATCHED RELATED DONORS

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Matched related donor (MRD) bone marrow transplantation is the treatment of choice for pediatric patients with severe aplastic anemia (SAA); however, only 25% of patients will have an HLA-identical sibling. Alternative donor transplants may be an option for these patients, but such therapies have been associated with greater incidences of graft failures and graft-versus-host disease (GVHD). We retrospectively analyzed 32 pediatric patients who have undergone 34 hematopoietic stem cell transplants for severe aplastic anemia at our institution from April 1997 to April 2005. One patient had a MRD transplant followed by a matched unrelated donor (MUD) transplant, while another had an HLA-mis-

matched unrelated donor (MMUD) transplant followed by a transplant from a haplo-identical parent. Twelve patients received MRD transplants, whereas 18 patients received alternative donor transplants—11 MUD, 3 haplo-identical, and 4 MMUD. The median age at transplant was 9 years (range 1.5 to 18.4 yrs). For MRD transplants, the conditioning regimen most often utilized cyclophosphamide 50 mg/kg × 4 days and ATG 30 mg/kg × 3 days. For alternative donor transplants, the conditioning regimen most often utilized cyclophosphamide 50 mg/kg × 4 days, Campath 3-10 mg × 4 days (dependent upon patient weight) or ATG 30 mg/kg × 3 days, and TBI (single fraction 200 cGy for MUD; two fractions 200 cGy for MMUD). Nine alternative donor recipients received ATG in their preparative regimens, whereas 11 received Campath. GVHD prophylaxis was either FK506 or cyclosporine ± mini-methotrexate. The overall survival for MRD patients was 91.7% versus 80% for alternative donor patients at a median follow-up of 47 months (range 3 to 100 months). Of our 32 patients, there were 5 deaths: pulmonary failure with extensive, chronic GVHD (n = 1); poor graft function with infection (n = 1); and infection (n = 3). For patients receiving alternative donor transplants, the overall survival for the Campath group was 81.8% vs. 77.8% in the ATG group. None of the Campath patients developed extensive, chronic GVHD compared to 3/9 ATG patients. In conclusion, alternative donor transplantation using Campath or ATG in the preparatory regimens offers a curative therapy for pediatric SAA patients with survival similar to that of patients receiving matched sibling transplants. Although follow-up is shorter, Campath may be associated with a reduced incidence of extensive, chronic GVHD and further investigation is warranted.

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HLA-MISMATCHED/HAPLOIDENTICAL BLOOD AND MARROW TRANSPLANTATION IN 207 CASES OF LEUKEMIA—REPORT FROM A SINGLE TEAM

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Objective: HLA-mismatched related donor blood and marrow transplantation (BMT) is a suitable alternative approach in patients who lack a matched donor. We hypothesized that differences in BMT-outcome might be influenced by age, type of leukemia and feto-maternal tolerance. **Patients:** Here is the report on outcomes after mismatched related donor BMT for leukemia in patients aged 3-53 years, performed by a single team and transplanted from April 2002 to May 2005 and followed through June 2005. 65 (31%) had CML, 57 (28%) AML, 73 (35%) ALL and 12 (6%) MDS. 46% had early stage disease, 30% intermediate and 24% had advanced disease at BMT. 32(16%) of grafts were mismatched at a single HLA locus, 93 (45%) at 2 HLA loci and 82 (40%) at 3 HLA loci. All patients received a uniform protocol (BuCy2 + ATG) for BMT. **Results:** All of patients achieved full engraftment. Rates of acute and chronic GVHD were similar in children and adults, $P = .67$ and $P = .20$, respectively. The two-year probabilities of TRM were 19% and 22%, and those of relapse were 25% and 17% in children and adults, respectively. Two-year probabilities of LFS and overall survival (OS) were 55% and 62% in children, and 61% and 65% in adults, respectively. The two-year OS in patients who received graft from their mother, father, sibling and offspring were 61%, 66%, 76%, and 62% ($P = .40$), respectively. Disease status was a significant impact factor on the survival. Two years OS in early, intermediate, and advanced stage at transplantation was 77%, 60% and 45% ($P = .002$), respectively. Multivariate analyses resulted that in children with advanced leukemia and BMT with female donor were associated with higher risks of relapse, treatment failure and overall mortality. However, in adult recipients, BMT with a cousin or father donor was associated with higher treatment failure and overall mortality and ALL was associated with higher relapse rates. Higher CD3 dose reduced the TRM. **Conclusion:** Mismatched related BMT appears to be a feasible option in patients who lack a matched donor. The avoidance of female donors for children and use of sibling or maternal donor for adults is likely to present a better survival after BMT. Graft-versus-leukemia effect might play a role in the lower relapse rates seen in patients with AML/CML rather than lymphoid malignancies.

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A MATCHED PAIR RETROSPECTIVE COMPARISON OF ADULT UNRELATED AND SIBLING DONOR ALLOGENEIC STEM CELL TRANSPLANTATION (SCT) FOR AML

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Recent studies have shown nearly equivalent survival outcomes for unrelated donor (URD) SCT in CML compared to sibling donors. This study compared outcomes for a set of URD transplants for AML and a strictly selected matching set of sibling donor transplants. 105 adult histocompatible URD transplant recipients with AML aged between 16 and 59 years at transplant were selected from the ABMTRR database. 105 adult first 6/6 HLA-identical sibling transplants were selected, matched with the subjects for disease stage, sex and age as a control group. There was no significant difference between subjects and controls in the distributions of time from diagnosis to transplant, donor-recipient sex match, prior therapies, donor age or performance status. The transplants were carried out from 1992 to 2002 inclusive. The median follow up of live subjects was 4.4 years and for live controls was 6.3 years. There were 18 good risk (CR1) and 87 poor risk (>CR1) recipients in both URD and sibling groups. Five-year disease free survival (DFS) was not significantly different for good-risk URD and sibling donor recipients (62% vs 40%, $P = .20$), or poor-risk URD and sibling recipients (21% vs 25%, $P = .20$). In a multivariate Cox regression model, the independent risk factors for DFS were disease stage beyond CR1 ($P = .001$), age >49 ($P = .03$) and recipient CMV positivity ($P = .007$). Both neutrophil and platelet engraftment were significantly more rapid in the sibling group but transplant related mortality at 100 days was not significantly different. There was no difference in the cumulative incidence of acute GVHD grade II or above at 100 days. The cumulative incidence of chronic GVHD at 1 year post transplant was greater in good risk URD transplants compared to controls (84% vs 61%; $P = .04$) but not poor risk transplants. Relapse occurred in 28% of good risk URD subjects and 16% of siblings ($P = .30$) while in poor risk subjects this was 39% and 29% ($P = .20$). Adult URD allograft recipients for good and poor risk AML in this study had equivalent DFS probability to sibling recipients. This study provides a rationale for a larger prospective study of risk factors in allogeneic transplantation for AML. The ABMTRR is an important national data resource providing a large and readily accessible sample frame for retrospective studies.

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NEW TREATMENT MODALITY BY ADOPTIVE TRANSFER OF CHIMERIC CELLS FROM TWO DIFFERENT MHC MISMATCHED DONORS

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Background: Donor bone marrow transplantation (BMT) may promote chimerism in solid organ and composite tissue allografts. This study was designed to evaluate the rationale of immunotherapy with adoptive transfer of donor-specific cells originated from two different MHC mismatched donors and transplanted to the same recipients. **Methods:** Seven primary trimera were created across the MHC barrier from LBN ($RT1^n$) (n = 7) and ACI ($RT1^a$) (n = 7) donors to LEW ($RT1^l$) recipients (n = 7), under a 7-day protocol of αβ-TCRmAb/CsA therapy. Bilateral intraosseous BMT (70×10^6) was performed from LBN and ACI donors to left and right femur respectively. Fourteen secondary trimeras were created via adoptive transfer of MACS-sorted primary chimeric cells: double positive $RT1^n/RT1^a$ cells (9.0×10^6 to $24.0 \times$